WEST AFRICAN CACAO RESEARCH INSTITUTE

QUARTERLY PROGRESS REPORT

No. 39

TAFO, GOLD COAST.

7 DEC 1955

WEST AFRICAN COCOA RESEARCH INSTITUTE
TAFO

THIRTY-NINTH QUARTERLY REPORT : JULY-SEPTEMBER, 1955

SENIOR STAFF

Director	: :	(Vacant)
Deputy Director	: :	A. Pickles (Acting Director)
Secretary	: :	G. H. Neal

TAFO STATION

Division of Entomology	: :	H. R. Mapother (on leave 14th September) F. Raw D. J. Taylor (on leave) P. B. Cornwell F. E. Decker P. F. Entwistle (arrived 6th September)
Division of Pathology	: :	W. T. Dale (on leave) A. L. Wharton G. Usher (returned 13th July) A. Attafuah
Division of Botany	: :	J. F. Archibald (on leave) H. H. Rogers (on leave) A. D. McKelvie
Division of Soil Science and Chemistry	: :	S. N. Adams E. Margaret Holden T. A. Rohan
Division of Agronomy	: :	R. J. Benstead R. Wickens (on leave 6th July)
Station Manager	: :	S. E. Ntim-Kwafo (on leave)

IBADAN SUB-STATION

Acting Officer-in-charge	:	J. Nicol
Entomologist	: :	R. G. Donald (on leave)
Pathologists	: :	R. M. Lister J. M. Thresh (on leave)

STAFF MATTERS

Mr. H.R. Mapother went on final home leave on 14th September after having spent three tours at the Institute on secondment from the Agricultural Research Council. Mr. P.F. Entwistle, who has been appointed to work on capsid problems, arrived at the beginning of September.

The Acting Director paid a short visit to the Ibadan Sub-Station in August. Dr. S.N. Adams spent several days there in September during which he examined and gave advice on the deficiency symptoms which become apparent on coppiced cacao under some conditions.

Mr. Pickles flew to England to attend the 1955 Cocoa Conference which was held at Grosvenor House, London from 13th to 15th September. Messrs. W.T. Dale, D.J. Taylor, and R.Wickens who were on leave at the time also attended the Conference. Mr. Pickles gave a paper on 'The Research Policy and Programme of the West African Cocoa Research Institute', and a paper by Dr. Adams and Mr. McKelvie on 'Environmental Requirements of Cocoa in the Gold Coast' was presented. Mr. H.H. Rogers who until recently was on the staff of the Institute gave a paper on 'New Varieties of Cacao in West Africa'.

VISITORS

Visitors during the quarter included Mrs. M. Ballinger (a Member of Parliament in the Union of South Africa), Mr. Harry Dodoo (General Manager of the Cocoa Marketing Board) with a party from Germany, and Dr. F. P. Mehrlich who was making a survey of the problems associated with cocoa production in the Gold Coast.

MANAGEMENT AND MAINTENANCE

The extensions to W.A.C.R.I. Club House were completed in July and the building was re-opened.

At Ibadan the construction of the new laboratories was started and good progress was made. The external structures of ten insectaries have been completed and seven of them are ready for use.

Average daily production and consumption of water was as follows :-

	Production	Consumption
July	19,165 gallons	18,671 gallons
August	21,586 "	20,996 "
September	20,667 "	18,486 "

Samples of water sent for analysis were reported to be free from pollution of any sort.

CROP DELIVERY

There are indications that the main Crop Season will be short and that most of the crop will be off the trees by mid-December.

There was very little flowering and setting during June, July and August.

Long periods of cool overcast weather during the quarter have been favourable for Black Pod Disease and as a measure of control more frequent harvesting is being carried out.

With abnormally heavy crop delivery in September and poor drying conditions, fermentation and drying facilities have been taxed to the utmost.

SWOLLEN SHOOT DISEASE

Station	July	August	September	Total
Old Station	28	43	34	105
Square Mile*	150	330	189	669
Adonkwanta	5	6	0	11
Akwadum	0	0	0	0
Nankese	0	0	0	0
Kukua	0	8		8
Koransang	0	0	0	0

* Including suspects and contacts.

Twenty two infected seedlings were removed on the Old Station.

OUT-STATIONS

Harvesting on all out-station farms, commenced in early September and crop prospects are good.

The programme of re-habilitation of areas heavily damaged by capsid, undertaken by the Entomology Division at Nankese has been completed.

Capsid damage has been confined to areas of poor establishment and broken canopy.

The Upper Amazon plots on the poor sandy soils at Akwadum have commenced to bear and although the vigour of the trees is poor by normal standards for this type of cocoa, establishment is complete.

RAINFALL

Monthly rainfall at Tafo and the out-stations is given below.

Station	Rainfall in inches				Wet days			
	July	Aug.	Sept.	Total	July	Aug.	Sept.	Total
Tafo Station	6.81	2.57	6.44	15.82	18	16	21	55
" 17-yr mean	5.39	2.62	2.24	14.25	13.6	12.8	14.3	40.7
Adonkwanta	8.79	1.77	3.90	14.46	11	8	8	27
Akwadum	4.59	2.90	5.76	13.25	11	12	15	38
Nankese	4.47	1.55	4.42	10.44	8	8	12	28
Kukua	4.62	1.05	3.62	9.29	8	6	8	22
Kerasang	5.29	1.45	3.31	10.05	7	6	6	19

TAFO STATION

1. VIRUS RESEARCH

(a) Host Range of Cacao Viruses

The survey of the distribution of Adansonia digitata L. has made good progress and records for the Eastern Region of the Gold Coast are now complete. A total of 150 trees in this area were located and examined for virus symptoms but no infected specimens were found. Further infection of Adansonia, on the Accra Plains, has been confirmed within a large block of trees south of the Shai Hills.

The survey for possible virus infection in Sterculia rhinopetala K. Schum. has continued. Material has been collected from trees in the New Juaben area around Tafo. So far, two have given a mild, transient form of red mottling in cacao seedlings used for the routine "back-tests". Results of the "back-tests" from most of the samples taken are not yet available.

In laboratory tests two more species of the Order Tiliales, Abroma augusta L. and Sterculia laevis Wall., have proved susceptible to infection with the New Juaben cocoa virus. A. augusta is a fibre plant and any extension of its cultivation in the Gold Coast would be important due to the potential danger of the crop becoming a source of virus infection. Material of this species came from Ejura near Kumasi. Seed of S. laevis was sent from Malaya; the species does not occur in the Gold Coast. Adansonia digitata is susceptible to the Alaparun strain from Nigeria. Work on the susceptibility to infection of plants within the family Malvaceae has started.

(b) Insect Transmission Studies

Pseudococcus njalensis Laing has been shown to transmit the Peki cacao virus.

(c) Classification of Viruses and Virus Strains

Tests to determine the relationship between Adansonia virus AD.7 and the Kpeve cacao viruses are being repeated. Further protection tests between the cacao viruses have shown that the Kpeve virus in the Gold Coast is not related to the Asalu isolate from Nigeria.

In studies, described in the 38th Quarterly Report, on mild-strain protection within the New Juaben virus complex, one of the 36 "protected" plants has shown virulent symptoms. The symptoms appeared after the plant had been "bombarded" nine times, at monthly intervals, using single mealybugs on each occasion.

The classification of the cacao viruses and virus strains included in the W.A.C.R.I. collection is receiving more attention. Apart from information obtained from cross-protection tests, latent period of the viruses in cacao and their host range, symptom patterns will be recorded visually and with the aid of photographs in colour.

(h) Resistance to the New Juaben Virus

Of the three plants, reported in the 38th Quarterly Report to be still symptomless after a second bombardment with 200 infective "crawlers" per plant, one is now showing evanescent leaf symptoms and all have stem swellings. The three plants had been back-tested for latent infection before the symptoms appeared. The results of these tests showed a high rate of infection to, and severe leaf symptom expression on, the Amelonado cacao test plants.

Bombardments in the first replication to compare resistance in rooted cuttings from 50 Iquitos-type trees are completed. Work on the second replication is in progress.

2. MEALYBUG STUDIES

(a) Movement of Mealybugs in the Canopy

Experiments to investigate the movement of mealybugs on infested cacao trees have continued. Trapping during successive periods throughout the day has shown that mealybug movement commences at about 11 a.m., reaches a maximum between 2 and 3 p.m. and falls rapidly towards sunset. A comparison has been made of the numbers of insects moving at different elevations on cacao trees with the position of the static population. Contrary to the statement made in the 37th Quarterly Report, "that movement is mainly confined to the canopy branches" more recent work has shown that there is a tendency for the downward movement of the population. Further work was started in the laboratory to investigate the relationship between mealybug movement and atmospheric factors.

Further experiments in the field were commenced to study the canopy movement of mealybugs between trees. In a preliminary experiment some 10,000 bugs were released on a single tree; catches made on the first and second rings of contact trees were less than 2% of the number of moving insects recorded on the release tree.

(b) Aerial dispersal studies

All experiments described in previous reports have been continued. The numbers of insects recorded on sticky traps have increased slightly within the period under review. The results of continuous trapping for over 12 months at 7 elevations from ground level to 30 feet above ground suggest that mealybugs are most readily dispersed by wind at levels below the canopy. Two new aerial traps were erected within the quarter. One consists of a tower supporting 800 cacao seedlings at various elevations. The other consists of 20 individual traps with fresh cacao twigs as bait at 20 elevations from ground level to 80 feet above ground. Preparations are being made for the examination of wind movement in cacao using insecticidal smokes.

(d) Observations on winged ants to determine to what extent mealybugs are carried on mating flights have continued.

(f) Mass-breeding of Mealybugs

Work on the development of a suitable technique for mass-rearing mealybugs has continued.

Under the controlled conditions mentioned in a previous report successful establishment of cacao seedlings was obtained. Young seedlings were bombarded with crawlers and adult mealybugs, care being taken not to introduce parasites and predators. In some cases cacao beans were inoculated with adult mealybugs between the cotyledons before planting but subsequent to germination the plants were found to be mealybug-free. On seedlings which were bombarded, however, colonies built up rapidly, but in the absence of ants they became smothered by moulds which developed on the honeydew. Both Crematogaster and Pheidole ants failed to establish and showed no apparent interest in the bugs.

The success of a technique for the mass-rearing of mealybugs is dependent on providing favourable conditions for the host plant, mealybug and associated ants. So far such ideal conditions have not been obtained. In the second phase of this work it is intended to concentrate primarily on determining the ideal conditions for ant establishment using potato as the host plant for the bugs.

3. STUDIES ON BLACK POD DISEASE

(a) Fungicide Control Trials

The harvesting figures for Trial I, given in the 38th Quarterly Report, suggested a marked reduction in the incidence of black-pod disease as a result of the fungicide spraying treatments. This control has been confirmed in the current quarter, when the disease rose to a high level on the unsprayed control plots.

The harvesting records and pod disease classification figures are tabulated below :-

TREATMENT		PODS PER TREE								
Fungicide	Frequency of application	Total	Damaged	%x	'Black-pods'	%x	Rodent	%x	Otherwise damaged	%x
Control (Unsprayed)	-	16.15	10.52	65.1	7.48	46.3	1.99	12.3	1.05	6.
'Carbide-Bordeaux'	Frequent	12.48	3.06	24.5	0.79	6.3	1.60	12.8	0.67	5.
	Monthly	13.58	4.75	35.0	2.03	14.9	1.87	13.8	0.85	6.
Bordeaux Mixture	Frequent	17.92	6.27	35.0	2.73	15.2	2.63	14.7	0.91	5.
	Monthly	14.22	6.06	42.6	2.89	20.3	2.19	15.4	0.98	6.
'Perenox'	Frequent	12.84	4.87	38.0	1.83	14.3	2.13	16.6	0.91	7.
	Monthly	9.33	4.68	50.2	2.89	31.0	1.13	12.1	0.66	7.

x Classified pods as a percentage of total pods harvested.

"Frequent" applications of the three fungicides (which involved a spray schedule based on the seasonal incidence of the disease and included weekly spraying in May, June and July) have given a better control of black pod disease compared, in each case, with the fungicide applied at monthly intervals.

So far this year Carbide-Bordeaux misture is giving the greatest control of the disease, the best treatment being this mixture applied under the "frequent" schedule, which reduced the percentage of the total pods attacked by the disease from 46.3 in the control plots to 6.3 in the sprayed. There appears to be no effective difference between the Bordeaux mixture and the Perenox treatments though the monthly sprayed figures in the table suggest that Bordeaux misture has a better residual effect than Perenox. However, the percentage "black pod" figure for monthly applications of Perenox includes records from one plot which showed no reduction in the disease. It was confirmed that this plot was being sprayed. From the field spot-plans of the experiment it was seen that the plot of forty-eight trees, unlike any other, was completely surrounded

by trees in control, unsprayed plots with a very high incidence of black pod disease. It is possible that the Perenox applied to the plot every month was not sufficient to counteract the disease spreading from the surrounding sources of high infection.

Weekly harvesting has also reduced the losses of pods from black pod disease compared with monthly harvesting. The actual figures ("black pods" expressed as percentages of the total pods harvested) for the two harvesting treatments, from the control and sprayed plots, are as follows :-

	Control (Unsprayed)	'Carbide-Bordeaux'	Bordeaux Mixture	'Perenox'
Weekly Harvesting	39.7	8.0	14.1	16.6
Monthly Harvesting	56.2	15.1	20.8	29.4

Pod losses due to the depredations of squirrels and rats are again high. There is no further evidence to suggest that losses are greater on the sprayed plots where at any one time there are more healthy pods on the trees susceptible to rodent attack.

No results for Trial II were given in the 38th Quarterly Report as few pods were harvested during the period covered. The main categories of pods harvested during the five months from May to September are summarized in the following table :-

TREATMENT		PODS PER TREE								
Fungicide	Frequency of application	Total	Damaged	%*	'Black pods'	%*	Rodent	%*	Otherwise Damaged	%*
Control (Unsprayed)	-	14.02	6.85	48.9	5.15	36.7	1.12	8.0	0.58	4.2
'Perenox'	Biweekly	16.08	4.95	30.8	2.61	16.2	1.63	10.1	0.71	4.5
	Monthly	10.92	4.36	39.9	3.01	27.6	0.97	8.9	0.38	3.4
'Blitox'	Biweekly	17.14	6.82	39.8	3.90	22.8	1.96	11.4	0.96	5.6
	Monthly	10.82	4.05	37.4	3.09	28.6	0.71	6.6	0.25	2.2

* Classified pods as a percentage of total pods harvested.

Some measure of control of black pod disease has been achieved by all the fungicide treatments and taking into account the longer period under review the effect of Perenox is very similar to the results obtained with this fungicide on Trial I.

The effect of weekly harvesting in reducing the numbers of "black pods" is shown below in a table comparable with the one given above for Trial I.

	Control (Unsprayed)	'Perenox'	'Blitox'
Weekly Harvesting	28.0	16.6	24.1
Monthly Harvesting	45.9	24.5	25.8

The mean percentage reduction in the numbers of pods attacked by the disease as a result of harvesting at weekly intervals was 32.1 for Trial I and 27.1 for Trial II.

(c) Cushion Infection

In the experiment to find out what proportion of proximal pod infections originate from diseased cushions (38th Quarterly Report) 1,234 pods have been harvested to date, from the 72 trees. Of these 760 came from the "accessible" parts of the trees and had been brush-painted weekly with a strong paste of Perenox; 8 of them showed distal "black pod" infections, 64 were attacked at the proximal end. The fungicide painting has almost completely prevented distal pod infections arising from spores of Phytophthora palmivora Butl. It is too early to say if the apparently large reduction in proximal pod infections is a true one (if it is, most of the infections have probably arisen from germinating spores and not from mycelium within the cushions) until the complete seasons records are available.

4. CAPSID STUDIES

(a) Capsid Pocket Regeneration Experiment

This experiment is proceeding satisfactorily and regeneration and growth of the trees is being recorded. Assessment of the fungi present in occluded lesions found within the trunks of coppiced trees is being undertaken by Mr. Usher.

Persistence of Insecticides

The persistence of DDT sprays applied to cacao foliage is being studied under field conditions by exposing adults of Tribolium castaneum Hbst. on the foliage under standard conditions and recording the percentage mortality. Results of one series of tests carried out between July 13th and August 24th on young trees growing under normal shade are summarised in the table.

Percentage mortality of T. castaneum Hbst.

DDT Emulsion	Interval after spraying			
	1 day	14 days	28 days	42 days
5%	100	86	74	28
2.5%	88	38	30	2
1.25%	50	19	6	0

These preliminary tests are being repeated under different environmental conditions and comparison is being made between the persistence of DDT and benzene hexachloride.

(f) Quantitative study of Capsid Eggs

The capsid infestation of the plots set aside for this study has decreased greatly and little progress has been made with the investigation.

(g) Investigation of fungi associated with capsid lesions

This work has been in abeyance for some time and investigations have been re-opened to determine the relative importance of the fungi that are found in capsid lesions.

93 lesions associated with capsid damage on young branches and chupons of cacao have been examined by the following technique. The lesion, or part of it, was removed with a sterilized scalpel and placed on Cassava dextrose agar in a petri dish. The dishes were placed in full daylight for 48 hours and the fungi growing from the wood examined and, as far as possible, identified. The results are shown below :-

Percentages of different groups of Fungi associated with capsid damage on cacao

FUNGUS	LESIONS			
	*Young	Medium	Old	Total
Fusarium decemcellulare Brick		1.1		1.1
+Fusarium spp.	7.5	7.5	6.5	21.5
Botryodiplodia theobromae Pat.	10.8	6.5	9.7	27.0
Other fungi	31.5	11.8	12.0	55.3
Uninfected	10.8	3.2	3.2	17.2

"*Young" = newly formed lesions.

"Medium" = lesions from newly formed up to time that phloem fibres appear separated.

"Old" = from the medium stage until they begin to become occluded.

+This category contains at least 7 species or sub-species.

22.1% of the lesions gave more than one fungus, and Phytophthora palmivora Butl. was never isolated. These experiments are continuing and similar ones have been started on occluded lesions from main stems.

Studies on the dissemination of these fungi have begun by examining the possibility of the capsids themselves being the vectors. 143 capsids (Sahlbergella singularis Hagl. and Distantiella theobroma (Dist.) have been collected in the field and exposed for half-an-hour on petri dishes of cassava dextrose agar. The plates were then treated as described in the previous experiment. 6.3% of the capsids carried F. decemcellulare 15.4% carried other Fusarium species while 13.6% carried B. theobromae. 99% of the insects were vectors for other fungi, a large proportion of which were Penicillium spp. and Aspergillus spp.

An experiment to determine whether the length of time the capsids were on the agar influenced the number of spores deposited showed, that leaving the insects on the plates for two hours had no advantage over the initial half-an-hour treatment. It is highly unlikely that there is any association between a particular species of capsid and any particular fungus.

5. EXPERIMENTS ON ESTABLISHMENT AND MAINTENANCE

(a) Establishment Trials

Routine maintenance has been carried out. Yields are being recorded for final analyses at the end of the season.

(b) Spacing Trials

Routine maintenance and recording of crop have been the only activities.

(c) Shade Trials and observations

In order to evaluate the effect of oil palm shade on yield, 20 plots each consisting of 18 trees have been selected under both Oil Palm and mixed forest shade at Nankese. Yields are being recorded in terms of pods and wet weight of beans.

(d) Comparative yield trials of seedlings and rooted cuttings

Routine maintenance has been carried out, shade has been reduced where too heavy.

All vacancies have been filled with well grown plants.

(e) Effect of Soil type on pod development and yield

Harvesting and recording continue.

(f) Method of forecasting yields of cocoa

Recordings continue.

(g) The effect of retention and removal of 'chupons' on rooted fan cuttings

Routine maintenance only has been carried out. No further losses are reported, but growth is slow.

(h) Frequency of Picking Experiments

The schedule as laid down is being strictly adhered to.

6. SOIL FERTILITY AND MANURIAL REQUIREMENTS

(a) Effect of Amounts and Combinations of Major Nutrients

(i) Routine fertilizer applications have been made on all existing trials. Height of seedling measurements have shown small but significant responses to super-phosphate at Poanu, Goaso and Juaso.

Shade removal of sections of the trials on Blocks A and H, W.A.C.R.I. and Bunso is now almost completed. This removal has produced an increase in vegetative growth but no fertilizer responses have as yet appeared.

(ii) No fertilizer responses have been observed in these experiments.

(iii) The second year's pre-treatment yield recording on these plots has been started by the Department of Agriculture. A tour of inspection of all sites was made in September and the harvesting methods checked.

(b) Effect of Different Sources of the Major Nutrients

No fertilizer responses were observed on these plots in the 1954-55 crop year.

(g) Investigation of Factors Limiting Growth in the Early Life of Cacao Seedlings

Shade removal from two of the four blocks of this experiment is now almost completed. A striking response to light has been obtained. A sample of seedlings removed as thinnings in August showed that plants in the lightly-shaded blocks had a dry weight 83% greater than those in normal shade. Moreover, a significant response to grass mulching has been observed in the lightly shaded blocks but not in normal shade.

(h) Role of Minor Elements

The 1st 10-acres on the Square Mile have been surveyed to estimate the extent of sickle-leaf disease in the area. It has been found to be widespread and appears to be depressing yield. In a few cases there is almost complete defoliation of the tree and the incidence of cherelle wilt is 100%. Attempts are now being made to remedy the zinc deficiency which is suspected to cause sickle-leaf. Leaf and soil samples from this area have been sent to the United Kingdom for analysis for minor nutrients.

There is still no sign of sickle-leaf on the trees which were liberally dosed with rotted wood ten months ago. Rates of applications are being increased.

7. VEGETATIVE PROPAGATION

Alternative rooting media are being tried to replace palm fibre which is sometimes in short supply. These include composted sawdust, elephant grass and rotted wood, each mixed with sand. It is too early yet to report on their value.

Good results are still being obtained using polythene sheeting over open beds of soils. Further supplies of sheeting are awaited before experiments are expanded in scope.

8. PLANT BREEDING AND SELECTION

In the absence of both Plant Breeders the work has had to be almost entirely routine.

(a) Established Trials

Harvesting was heavy during the quarter. The 8th P.T.A. is now coming into bearing after 3 years in the field

and 133 pods have been harvested already this year. The Upper Amazon types are the highest yielders with the hybrid crosses close behind. Girth Measurements for the 9th P.T.A. are shown below :-

9th P.T.A. Stem Diameter (cm.) June 1955.

TF 1	WE 6	WAE11	WBE6	WEA2	WEA3	WEA4	WEB1	WEB2	WEB3
1.54	2.05	2.20	1.90	1.94	1.95	2.14	2.38	2.17	1.89

0.1% 2,4D (ammonium salt) was successfully used in one of the trials as a herbicide. A survey of the species susceptible to the 2,4D is being made. There was no damage to the cacao trees. Treatment was applied on 9th August and while adjacent brushed plots at the end of September are needing further brushing, the plots sprayed with 2,4D are still clear.

(e) Inter-specific crosses

Pods from crosses between Herrania and Theobroma have been harvested and are being examined, especially for fat and alkaloid content.

(j) Storage of Pollen

Experiments have begun to test the viability of pollen after storage at low temperatures. No success has been achieved yet but attempts are continuing.

9. CACAO FERMENTATION AND QUALITY

(a) Fermentation and Chemical Constitution

Fermentation

Several single pod fermentations have been carried out under varying conditions using West African Amelonado, the results cannot be assessed until the small chocolate making laboratory is complete.

A study of all the normal methods of bulk fermentation has been started. It is intended to record temperature variations, quantitative estimations of polyphenolic compounds, and the appearance of the cotyledons with particular reference to purple pigments, during fermentation, drying and subsequent storage.

Identification of the Catechins in West African Amelonado

By chromatography on a column of powdered cellulose (Solka Floc.) using the solvent layer of amyl alcohol-acetic acid-water (4:1:5 v/v) and examining the effluent by paper chromatography four catechins were identified by their R_F values in the three solvent systems; butanol-acetic acid-water (4:1:5); amyl alcohol-acetic acid-water (4:1:5) and phenol-water (1:1).

	BUTANOL-ACETIC	AMYL-ACETIC	PHENOL
No.1	0.66	0.42	0.50
No.2	0.74	0.52	0.39
No.3	0.58	0.28	0.27
No.4	0.48	0.23	-

Comparison of the above figures with established R_F values for catechins shows that No.1 corresponds with (-)-epigallocatechin, No.2 with catechin, No.3 with gallocatechin and No.4 with epigallocatechin.

Quantitative Paper Chromatographic Analysis of the Polyphenols

Two dimensional chromatography of an acid extract of ripe Amelonado beans first with water and then with the solvent layer of butanol-acetic acid-water (4:1:5) gave a chromatogram in which all the main anthocyanins, leucoanthocyanins and catechins appeared as discrete spots on spraying with a solution containing equal volumes of ferric chloride and potassium ferricyanide solutions. The distribution of the polyphenols on this chromatogram was similar to that found by Forsyth in the case of I.C.S.I. cocoa and it was again evident that the use of water as a developing agent separated the anthocyanins, catechins and leucoanthocyanins into clearly defined composite groups in descending order of mobility.

0.5 ml. of an extract of ripe Amelonado beans (10 g. beans in 5 ml. 0.3N hydrochloric acid) was applied to a sheet of Whatman No.1 paper as a streak 22 cm. in length and developed with water until the solvent front had travelled 30 cm. After drying, the chromatogram was cut into strips 1 cm. wide and each strip, in 1% sulphuric acid (10 ml.), titrated against 0.01N potassium permanganate. A graph of ml. permanganate against strip number, measured from the origin, gave distinct peaks at the four main classes of polyphenols: anthocyanins, catechins and the two leucoanthocyanin groups.

The application of Forsyth's technique to West African Amelonado (private communication) has shown that the method might be applied to the study of the changes in the polyphenols which take place during fermentation, drying and storage and work has just been started on this project.

Purple Beans

The two principal anthocyanins have been isolated on a column of powdered cellulose (Solka Floc.) and it has been shown by acid hydrolysis of the two anthocyanins that they are both derived from cyanidin, which was identified by colour reactions (Robinson & Robinson, Biochem. J., 1931, 25, p.1687) and its R_F (0.69) when chromatographed in butanol-2N hydrochloric acid (Bate Smith, Biochem. Soc. Symposia, 1949, 3, p.62).

Acid hydrolysis of each pigment and paper chromatography of the hydrolysate has shown that the glycosidic residue in both is glucose: the pentose in the pentose glycoside AII has not yet been identified.

MacLean (Quarterly Progress Report No.33, 1954) reported that "the purple pigment in fresh, unfermented beans appears to consist of a purple residue in addition to the two anthocyanins indicated." Forsyth (Nature, 1949, 164, p.25) had already shown that in addition to the main pigments there existed also a cyanidin diglycoside but that this was only a minor constituent.

In order to determine whether the ordinary method of acid extraction results in preferential solution of the more soluble pigments leaving the less suluble in residue, an exhaustive extraction of fresh unfermented Amelonado cocoa with dilute hydrochloric acid was carried out. A completely colourless residue was obtained, the final

extraction being made with methanol. Chromatography of the extracts on paper with butanol-acetic acid showed only the presence of the two main pigments with a small amount of coloured immobile material which remained on the starting line - probably Forsyth's diglycoside.

It would appear therefore that there are no coloured pigments other than those already reported and that although one acid treatment results in incomplete extraction of the anthocyanins all are taken up probably in proportion to the extent of their occurrence in the bean.

The colourless residue, on boiling with 20% hydrochloric acid, gave cyanidin. Hydrolysis with 0.5N sulphuric acid (Forsyth, Biochem. J. 1952, 51, 511) gave a solution of sugars as indicated by a positive benzidine reaction. Paper chromatography with butanol-acetic acid gave two spots (R_F 0.27 and R_F 0.33) on spraying with ammoniacal silver nitrate. These could not be separated from glucose and arabinose respectively but separated readily from galactose, xylose and rhamnose.

The insoluble residue after complete extraction of the coloured pigments might therefore contain a leuco-anthocyanidin arabino glucoside which gives cyanidin and the two sugars on hydrolysis.

(b) Effect of Storage on Purple Bean Content

Fermented and dried cacao has been deposited at Cadbury & Fry warehouse throughout the Coast and Ashanti. Humidity and temperature records will be kept and the beans sampled every two months and the moisture and purple bean content determined.

A bag stored at Tafo will be used for a more detailed study of the changes taking place on storage.

(c) Survey of Farmers' fermentation methods

There will be nothing to report until samples are received from selected farms.

Reports from areas indicate that these may be expected in October.

12. PHYSIOLOGY OF NUTRITION, GROWTH AND FRUITING

(e) Investigation of cherelle wilt

(i) It has been confirmed that α-naphthalene acetic acid (NAA) is of no value in preventing cherelle wilt even at 7 and at 10 weeks after pollination when pods are specially susceptible to wilt. NAA at 1000 p.p.m. does seem to cause thinning of young cherelles but it is too early in the season to say whether this will increase yield as is the case with thinning sprays on some temperate fruit crops.

Pods have been sprayed with NAA and pod stalks have been treated with lanolin containing NAA but no differences in growth and wilting behaviour between treated and control pods were observed.

(ii) The results quoted in the W.A.C.R.I. Annual Report 1954-55 concerning the hormonal basis behind cherelle wilt have been repeated and confirmed and a full account of this work is in the press and will be published shortly.

(iii) In the experiment on flush prevention there has been so far this season a 90% increase in yield on trees where flushing has been prevented (by the hand picking of buds). These trees trees are being allowed to flush in the heavy flush which is beginning at the end of September, since the number of leaves on them is now only 40% of the leaves on control (untreated) trees.

Maleic hydrazide (0.1%) has been found to prevent flushing of young cacao seedlings and an experiment has begun to test its effect on trees in the field. While 0.1% NAA was reported previously (Quarterly Report No.34) to prevent flushing its effect only lasts for about 2 weeks and it causes rapid fall of mature leaves.

Suitable precautions are being taken in the maleic hydrazide experiment to avoid contamination of surrounding cacao and all ripe pods on treated trees are being destroyed until a report can be obtained as to the effect, if any, of this chemical on flavour.

(iv) Trio-iodo benzoic acid (TIBA) is being used in an effort to accelerate flowering of young trees. Applied as a 0.1% aqueous spray it inhibits terminal bud growth but no acceleration of flowering has yet been noticed.

(v) For future studies on the periodicity of cushion bearing in relation to cherelle wilt and black pod disease, an experiment has been started (in conjunction with the (Mycologist) in which all leaf axils on young trees are labelled and behaviour of the resulting cushions observed over a period of several years.

13. STUDY OF THE EFFECTS OF "SHADE" IN CACAO CULTIVATION

(a) Fertilizer Treatments in Relation to Shade Conditions

The NPK trial in pots in 50%, 80% and 100% light intensities has been harvested. The results have not yet been statistically analysed so no firm conclusions can be drawn.

(b) Effect of Forest Litter Removal

Routine work has been continued on this experiment.

(c) Effect of organic and inorganic mulches in relation to shade and fertilizer treatments

The layout of this experiment has been discussed in the W.A.C.R.I. Annual Report 1954-55. At the beginning of July the heights (in cm.) of plants in the various treatments were as follows :-

	Artificial shade	No shade	Natural shade
Sand	42.57	27.76	31.86
Mulch	57.27	39.87	41.34
Sand + N	47.10	28.40	31.91
N	56.65	32.69	33.30
Mulch + N	66.84	29.19	33.78
Control	42.90	39.35	35.28

(d) Comparison of Yields of Shaded and Unshaded Cacao

Yield recording on the four unshaded farms selected in the previous quarter has been commenced by the Department of Agriculture.

(f) Effect of day length and fertilizer treatment on growth of cacao seedlings

This experiment has been discontinued. Heights (in cm.) of the seedlings in July were :-

Hours of exposure	8.00-12.00	8.00-16.00	6.00-18.00	12.00-16.00
Treatment				
N	16.20	24.16	33.95	10.00
P	15.20	26.45	35.20	11.16
K	15.70	22.16	30.91	7.79
Control	16.33	24.29	29.16	9.12

The conclusions to be drawn are that growth is best under natural day length conditions and worst in a day length of 4 hours in the afternoon. 4 hours sunlight is better in the morning than in the afternoon. Phosphate gave a significant response in the 8- and 12-hour treatments.

IBADAN SUB-STATION

1. VIRUS RESEARCH

(b) Insect Transmission Studies

(i) Vectors of CSSV: no infections have been noted in experiments designed to test Geococcus coffeae as a vector of cacao virus.

(ii) Virus Movement: investigations into the rate at which CSSV moves through seedlings infected by mealybugs were started some months ago. So far it has been found that CSSV will move from the point of inoculation to a point at least 10 cm. away within 24 hours. If confirmed, this gives a rate of movement of at least 1 cm. in two and a half hours, which compares with that of viruses which it is thought must be injected directly into the phloem to cause infection, when these are transmitted to plants whose rate of translocation is low. (Bawden: "Plant Viruses and Virus Diseases" 1950 p.295).

(c) Classification of Viruses and Virus Strains

(i) Virus Collection: two more suspected outbreaks, including 16 trees in all, have been found in the Asalu area. They are several miles away from any of the others mentioned in the last Quarterly Report. Leaves collected from trees in each outbreak show the very characteristic symptoms of this type of infection, and a check for the presence of virus is being made using grafts to healthy seedlings. As pointed out in the last Quarterly Report the Asalu virus has never been transmitted experimentally other than by grafting.

(ii) Heat Treatment of Virus-infected Plants: investigations have been started into the effects on the symptom pattern and infectivity of plants infected with various isolates of CSSV and grown at a high temperature for long periods. Preliminary results have been discouraging, and with the techniques tried so far no change in infectivity has been apparent.

(iii) Effects of Virus Infection on the Growth of Seedlings: three replications of the experiment reported in the Quarterly Report No.37 have been set up. They will not be ready for recording and analysis for some weeks, but the seedlings are already showing differences in development according to the virus isolate with which they are infected.

(i) Coppicing Experiments

(1) INA Farm: the coppiced plots at INA Farm mentioned in previous Quarterly Reports continue to make good growth. No significant capsid damage has occurred except in Plot GO which is not included in the routine insecticidal spraying schedule, as observations are being made there on the insects colonising untreated developing chupons.

A considerable number of chupons have been damaged by insects feeding within stem tips, destroying the terminal bud and preventing normal jorquette formation. Besides Tragocephala sp., the larvae of a Noctuid moth Earias sp. have been found feeding within stem tips.

On the most recently coppiced plots, particularly the acre being used by the Cocoa Soil Survey Division of the Department of Agriculture for investigating the effects of mineral nutrients on the growth of chupons, some of the developing chupons are stunted and their leaves have fallen while still very small and undeveloped. The stipules are retained, and this gives rise to a characteristic "paint-brush" appearance, the chupons being short and straight with a few stipules along their length and a tuft of stipules at the tip.

It is thought that some environmental factors might be responsible, such as light, soil water content, soil compaction, or mineral deficiency related to seasonal effects. On the Soil Survey plot where all tree shade was removed, "paint-brush" is a serious problem.

(2) Owena: a high percentage of stumps in the coppiced plots at Owena have regenerated but the growth of the chupons is not vigorous. The trees were coppiced in June this year, and after 3 months growth are rather stunted. Iron deficiency symptoms occur on the majority of the chupons, and some show the "paint-brush" effect.

Before coppicing, all the trees were graded according to their canopy category using the arbitrary classification adopted in experiments at INA Farm, as follows:-

Category 0: complete canopy; no visible damage.

Category 1: complete canopy; damage to the tree visible only on close inspection.

Category 2: damage to the tree obvious but not extensive

Category 3: damage to the tree obvious, sections of the canopy showing die-back, dead leaves, or leafless, but damage not so extensive as to promote chupon development along the main stem.

Category 4: canopy largely destroyed, with extensive die-back and chupons growing along the main stem.

Category 5: main canopy totally destroyed, any foliage present due to chupon growth; in extreme cases the tree is alive at the base only.

From individual tree records of pod yields made available by the Federal Research Botanist it seems that pod yields are related to the canopy category, and also that for trees in high categories as recorded in 1955, yields have been low since 1949 (Table).

Total Pod Yields* for Trees in part of Owena Coppiced Area arranged according to Canopy Category

Canopy Category	Total Trees	1949-50		1950-51		1951-52		1952-53		1953-54		1954-55	
		Total Yield	Avg.	Total Yield	Avg.	Total Yield	Avg.	Total Yield	Avg.	Total Yield	Avg.	Total Yield	Avg.
0	46	413	9.0	732	15.9	446	9.7	695	15.1	499	10.8	671	14.6
1	67	445	6.7	1080	16.2	822	12.3	1161	17.4	864	12.9	1393	20.8
2	147	1077	7.3	2239	15.1	1397	9.5	2412	16.4	1635	11.1	2227	15.2
3	126	909	7.2	1799	14.2	1151	9.2	1793	14.2	1546	12.3	1356	10.7
4	276	1747	6.3	2648	9.6	1484	5.4	2247	8.1	1799	6.5	897	3.3
5	106	274	2.6	405	3.8	157	1.5	318	3.0	191	1.8	74	0.7

* Includes black pods and those damaged by animals.

(3) Araromi (Ogo): most of the 1200 trees coppiced at Ogo, near Araromi, in experiments to confirm that all infected trees produce symptom-bearing chupons are regenerating, and the chupons are being observed for the development of symptoms of swollen shoot disease.

SKB.